New insights into the chromosomal differentiation patterns among cichlids from Africa and Madagascar

by

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Abstract. – To increase understanding of the chromosomal diversity among cichlid species, karyotypes of 19 species of Cichlidae belonging to 15 genera (*Paratilapia polleni, Chromidotilapia guntheri guntheri, Hemichromis fasciatus, Steatocranus irvinei, Stomatepia pindu, Altolamprologus compressiceps, Julidochromis transcriptus, Neolamprologus tetracanthus*, *N. brevis, Gnathochromis permaxillaris, Eretmodus cyanostictus, Astatoreochromis alluaudi*, *Haplochromis callipterus*, *H. paludinosus*, *Nimbochromis livingstonii*, *N. polystigma*, *Pseudocrenilabrus multicolor victoriae*, *P. philander* and *Ctenochromis horei*) were investigated for the first time. In spite of the variation in the karyotypes of cichlid fishes (from 2n = 42 to 2n = 48 chromosomes), the modal chromosome number was 2n = 44. Cytogenetic mapping of the 18S ribosomal RNA (18S rRNA) gene was accomplished.

Résumé. – Nouveaux aperçus dans la différenciation chromosomique parmi les cichlidés d'Afrique et de Madagascar.

Afin de contribuer à une meilleure connaissance de la diversité chromosomique chez les cichlidés, les caryotypes de 19 espèces de Cichlidae appartenant à 15 genres (*Paratilapia polleni, Chromidotilapia guntheri guntheri, Hemichromis fasciatus, Steatocranus irvinei, Stomatepia pindu, Altolamprologus compressiceps, Julidochromis transcriptus, Neolamprologus tetracanthus*, *N. brevis, Gnathochromis permaxillaris, Eretmodus cyanostictus*, *Astatoreochromis alluaudi*, *Haplochromis callipterus*, *H. paludinosus*, *Nimbochromis livingstonii*, *N. polystigma*, *Pseudocrenilabrus multicolor victoriae*, *P. philander* et *Ctenochromis horei*) ont été établis pour la première fois. Malgré la variation des caryotypes des cichlidés dans le nombre de chromosomes (de 2n = 42 à 2n = 48 chromosomes), le nombre modal de chromosomes a été confirmé (2n = 44). La localisation de l'ARN ribosomique (ARNr) 18S a été identifiée par hybridation *in situ* en fluorescence (FISH).

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Among teleosts, cichlid fishes are well known for their speciose adaptive radiations in the Great Lakes of East Africa. This monophyletic lineage diversified over 9-12 million years (Myr) in the Lake Tanganyika (Cohen *et al.,* 1993), the most ancient lake (Tiercelin *et al*., 1989) in Africa, and within 2 Myr in the Lake Malawi (Fryer and Iles, 1972) and 12400 years in the Lake Victoria (Johnson *et al*., 1996). Because of their striking species richness, their morphological and ecological diversity and their high rate of endemism, they have been a prime model system for the study of speciation in evolution (reviewed in Meyer *et al*., 2015). Due to their outstanding diversity in colour, morphological and behavioural patterns, cichlid species have become popular as aquarium fishes. Tilapiine cichlids are extensively used for food in aquaculture and are actually a predominant cultured species worldwide according to the FAO (2014). In order to shed light on the mechanisms leading to their striking phenotypic diversity, numerous investigative approaches such as comparative anatomy, behavioural and population investigations, genetic, phylogenetic and molecular studies, have been undertaken. Recently, the genomes and transcriptomes of five species representing five lineages of East African cichlids have been sequenced and annotated (Brawand *et al*., 2014).

Complementary to all these approaches, cytogenetics has been utilized: 1) to establish karyotypes, so as to tentatively point out the main chromosome changes occurring during the evolutionary history of this family (reviewed and updated in Poletto *et al*., 2010); 2) to physical mapping by FISH (fluorescence *in situ* hybridization) genes of interest (Cnaani *et al*., 2007; Lee *et al*., 2010) in the chromosomes; 3) to obtain a global view of the chromosomal distribution

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and abundance of some particular sequences (Ferreira and Martins, 2007; Valente *et al.*, 2011) and 4) after construction of a high-resolution radiation hybrid (RH) panel for the Nile tilapia, to confirm orientation and assignation of the RH groups to the corresponding chromosome, which can be identified in the karyotype (Guyon *et al.*, 2012).

More than 2000 cichlid species have been described, mainly from Africa and South America (Sparks and Smith, 2004), but few of the African species (around 40, according to Poletto *et al.*, 2010) have as yet been karyotyped.

As a baseline for further cytogenomic investigations, we present here the unpublished karyotypes (including the chromosomal mapping of the 18S ribosomal RNA genes) of nineteen species of the African subfamily Pseudocrenilabrinae. In addition, we provide similar data for one species from Madagascar, belonging to the subfamily Ptychochrominae. Our results are then compared with those already available within each investigated tribe. They confirm that chromosomal rearrangements, as well as 18S rRNA gene positions, cannot be used for clarifying phylogenetic relationships among the different cichlid clades. Indeed, convergent rearrangements occurred separately in the different lineages. However, it is possible to delineate major chromosomal characteristics for some lineages.

MATERIALS AND METHODS

Chromosome preparation and specimens under study

Metaphase chromosomes were obtained from cephalic, kidney and spleen tissues by direct *in vivo* method, according to the protocol of Bertollo *et al.* (1978) with the following modifications: fishes were euthanised with a lethal dose of ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich) prior to dissection. Cephalic, kidney and spleen tissues were rapidly dissociated with forceps, on 70 μm mesh cell strainers placed in small Petri dishes containing 2 ml hypotonic solution (0.075M KCl). The cell suspension was transferred into 15 ml conical tubes containing 10 ml of pre-warmed hypotonic solution. The hypotonic treatment was performed 20 min in a water bath at 23°C with regular gentle agitation. The cells were fixed three times in 3:1 methanol-acetic acid and spread by air-drying onto clean slides. The animal experiments were granted by the Ethics Committee of the Ministry of Environment.

The list of specimens studied, their vouchers and their locality of capture are provided in table I and figure 1.

Chromosome numbers and formulae

Mitotic chromosomes were DAPI (4',6-diamidino-2-phenylindole) stained and karyotypes were established according to the centromere position following the nomenclature of Levan *et al.* (1964). The chromosomes were classified into meta/submetacentric (m/sm) and acrocentric, including subtelo- and telocentric (st/t), hypothetical pairs on the basis of their size and morphology (in absence of molecular analysis), and were organized by order of decreasing size in the karyotypes.

18S rRNA probe preparation

The 18S rRNA probe, a segment of approximately 1,400 base pairs (bp), was obtained by PCR with the primers UnivF-15 (5'-CTGCCAGTAGTCATATGC-3') and UnivR-1765 (5'-ACCTTGTTACGACTTTAC-3') (Frischer, 2000) from the nuclear DNA of *Oreochromis niloticus* (aquaculture strain obtained from CIRAD, Montpellier). Amplifica-

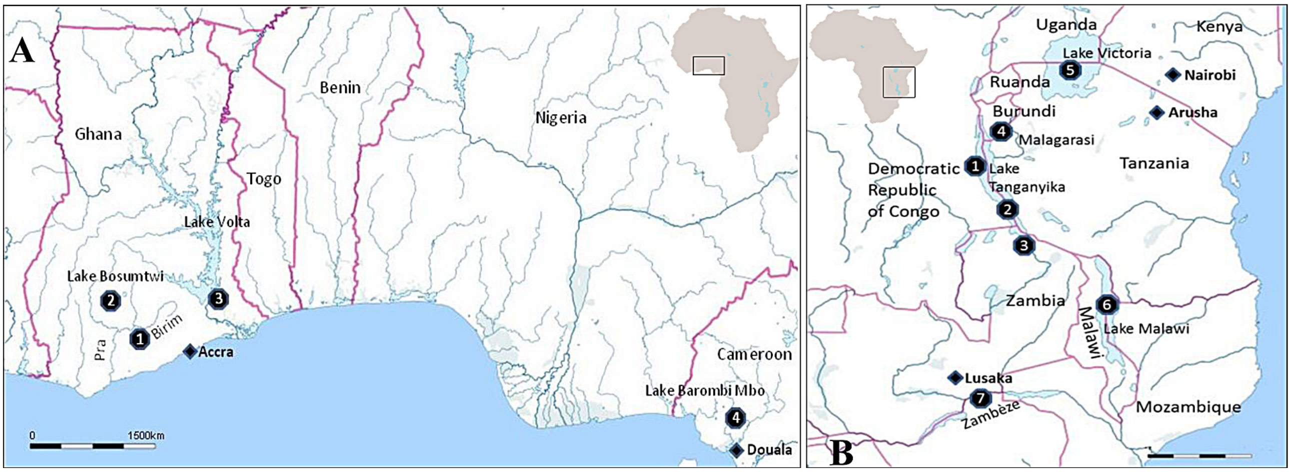

Figure 1. – Map of Africa showing the eleven collection sites of the cichlid specimens. **A**: West-central Africa. 1: *Chromidotilapia guntheri guntheri*; 2: *Hemichromis fasciatus*; 3: *Steatocranus irvinei*; 4*: Stomatepia pindu;* **B**: East central Africa. 1: *Altolamprologus compressiceps*, *Gnathochromis permaxillaris*; 2: *Ctenochromis horei*, *Neolamprologus tetracanthus*; 3: *Eretmodus cyanostictus*, *Neolamprologus brevis*; 4: *Haplochromis paludinosus*; 5: *Astatoreochromis alluaudi*, *Pseudocrenilabrus multicolor victoriae*; 6: *Nimbochromis livingstonii*, *Nimbochromis polystigma; 7: Haplochromis callipterus*. (Paugy et al., 2008).

Table I. – List of specimens analyzed, their vouchers and their origins. The species are distributed in subfamilies and tribes according to Dunz and Schliewen (2013) and Meyer *et al.* (2015), for tribes Chromidotilapiini and Hemichromiini according to Schwarzer *et al.* (2009). Undet. = sex undetermined. t/st = acrocentric.

Subfamily / Tribes	Genus / Species	Chromosome numbers	Chromosome formulae	Fundamental number	Specimens number male / female / undet.	Voucher	Geographic origin
Pseudocrenilabrinae							
Chromidotilapini	*Chromidotilapia guntheri guntheri*	48	48t-st	48	2/0/0	MNHN-IC-2002-3264	Ghana, Pra basin, Birim River
Hemichromini	*Hemichromis fasciatus*	46	6m-sm + 40t-st	52	0/3/1	MNHN-IC-2002-3261	Ghana, Lake Bosumtwi, Abonu
Steatocranini	*Steatocranus irvinei*	42	10m-sm + 32t-st	52	0/0/1	MNHN-IC-2002-1849	Ghana, Akosombo, Black Volta basin
Tilapiini	*Stomatepia pindu*	44	12m-sm + 32t-st	56	1/0/0	MNHN-IC-2004-1251	Cameroun, Lake Barombi Mbo
Lamprologini	*Altolamprologus compressiceps*	42	10m-sm + 32t-st	52	0/0/1	MNHN-IC-2003-0276	Congo, Lake Tanganyika
	Julidochromis transcriptus	42	10m-sm + 32t-st	52	1/0/0	MNHN-IC-2004-3156	Aquarium (lake Tanganyika)
	Neolamprologus tetracanthus	42	10m-sm + 32t-st	52	1/1/0	MNHN-IC-2004-0874	Lake Tanganyika
	Neolamprologus brevis	42	14m-sm + 28t-st	56	0/1/0	MNHN-IC-2004-1177	Zambia, Lake Tanganyika
Limnochromini	*Gnathochromis permaxillaris*	44	10m-sm + 34t-st	54	0/1/0	MNHN-IC-2003-0600	Congo, Lake Tanganyika
Eretmodini	*Eretmodus cyanostictus*	46	10m-sm + 36t-st	56	1/0/1	MNHN-IC-2004-0875	Zambia, Lake Tanganyika
Haplochromini - Thropheini	*Astatoreochromis alluaudi*	44	12m-sm + 32t-st	56	1/0/0	MNHN-IC-2004-3151	Lake Victoria
	Haplochromis callipterus	44	12m-sm + 32t-st	56	1/0/0	MNHN-IC-2002-3263	Zambia, Zambeze basin
	Haplochromis paludinosus	44	12m-sm + 32t-st	56	1/2/0	MNHN-IC-2002-3259	Burundi, Malagarasi River
	Nimbochromis livingstonii	42	14m-sm + 28t-st	56	0/1/0	MNHN-IC-2004-1179	Lake Malawi
	Nimbochromis polystigma	44	14m-sm + 30t-st	58	0/1/0	MNHN-IC-2003-0277	Lake Malawi
	Pseudocrenilabrus philander	44	16m-sm + 28t-st	60	0/1/0	MNHN-IC-2004-2302	Aquarium (East Africa)
	Pseudocrenilabrus multicolor victoriae	44	16m-sm + 28t-st	60	0/0/1	MNHN-IC-2004-3150	Aquarium
	Ctenochromis horei	40	10m-sm + 30t-st	50	1/0/1	MNHN-IC-2004-1176	Lake Tanganyika
Ptychochrominae	*Paratilapia polleni*	44	16m-sm + 28t-st	60	0/0/1	MNHN-IC-2004-0552	Aquarium, Madagascar

tion products were purified with GeneJET PCR Purification Kit (Fermentas Life Sciences), cloned in pGEM-T vector according to the manufacturer's recommendations (Promega) and sequenced (GATC Biotech AG). The probe was labelled by nick-translation with biotin-16-dUTP (Roche).

Fluorescence *in situ* hybridization (FISH)

The chromosome preparations were denatured at 72°C ± 2°C for 45 s in 70% (v/v) deionized formamide, 2 X SSC (pH 7), dehydrated through 70% (–20°C), 80% and 100% ethanol series at room temperature, 2 min each, and air-dried. Hybridizations were conducted with 15 μl of probe mixture (75 ng of denatured 18S rRNA probe) per slide under a 24 x 40 mm coverslip in a 37°C moist chamber for 24 h. Post-hybridization washes were carried out at 72°C in 2 X SSC for 5 min, followed by a second wash in PBD ("Phosphate Buffer-Detergent", 4 X SSC, 1% Tween 20), for 1 min at room temperature. Detection of the hybridized probe was carried out with 20 μg/ml of Cy3-Avidin conjugate (Sigma) in PBD for 5 min at 37°C. Slides were washed three times for 2 min each in PBD at room temperature. Chromosomes were counterstained with 1 μg/ml DAPI (Sigma) in antifade solution (Vectashield Mounting Medium, Vector Laboratories, Peterborough, UK).

Microscope analysis

Hybridized chromosomes were visualized using a Zeiss AXIO Imager M1 microscope, and images were captured with a CoolSNAP ES camera. Photomicrographs were processed by the Genus software for karyotyping and FISH-Imaging (Leica Microsystems).

Nomenclature and abbreviations used in the text

m: metacentric chromosomes; sm: submetacentric chromosomes; st: subtelocentric chromosomes; t: telocentric chromosomes; acrocentric: regroups st and t chromosomes, especially when chromosome arm condensation does not permit to identify as T or st; FN: fundamental number, arm number; NOR: nucleolus organizer regions.

Systematics

In the description of results and the following discussion, and because most species analysed herein belong to the East-African clade, the delineation of genera, tribes and subfamilies essentially follows the nomenclatures used in Dunz and Schliewen (2013) and Meyer *et al.* (2015); for the tribes Chromidotilapiini, Hemichromiini we used the nomenclature and delineation of Schwarzer *et al*. (2009).

RESULTS

In all species analysed, no occurrences of differentiated sex chromosomes were recorded.

Madagascar Cichlidae

Subfamily Ptychochrominae

Paratilapia polleni Bleeker, 1868

The karyotype of *P. polleni* is composed of 2n = 44 chromosomes, 16 m/sm and 28 st/t, including a large submetacentric pair and a large acrocentric pair (FN = 60) (Tab. I). The 18S rRNA genes are located on the terminal region of short arms of one acrocentric pair, the fourth one in size (Fig. 2).

African cichlid subfamily Pseudocrenilabrinae

Tribe Chromidotilapiini

Chromidotilapia guntheri guntheri (Sauvage, 1882)

The karyotype of *C. guntheri guntheri* is composed of 2n = 48 acrocentric chromosomes of slowly decreasing size, except the first pair, which is much larger. The most remarkable characteristic of *C. guntheri guntheri* karyotype is the exclusive presence of st/t chromosome pairs, (FN = 48). The 18S rRNA genes are located on the terminal region of short arms of one acrocentric pair, the second one of the size series (Fig. 2).

Tribe Hemichromini

Hemichromis fasciatus Peters, 1857

The karyotype of *H. fasciatus* is composed of 2n = 46 chromosomes including 6m/sm and 40 st/t (FN = 52) (Tab. I). The 18S rRNA genes, localized in the chromosomes of a single specimen, were found on two single acrocentric chromosomes of similar size and morphology and possibly homologues, one on the short arms, the other one in telomeric position, in the long arms (Fig. 2).

Tribe Steatocranini

Steatocranus irvinei (Trewavas, 1943)

The karyotype of *S. irvinei* is composed of 2n = 42 chromosomes including 10 m/sm and 32 st/t (FN = 52) (Tab. I). The 18S rRNA genes are located on the short arms of a small acrocentric pair (Fig. 2).

Tribe Tilapiini

Stomatepia pindu Trewavas, 1972

The karyotype of *S. pindu is* composed of 2n = 44 chromosomes including 12 m/sm and 32 st/t (FN = 56) (Tab. I). The 18S rRNA genes are located on the short arms of a small acrocentric pair (Fig. 2).

Tribe Lamprologini

We analysed the lamprologine species, endemic to Lake Tanganyika, *Altolamprologus compressiceps* (Boulenger, 1898)*, Julidochromis transcriptus* Matthes, 1959*, Neolamprologus tetracanthus* (Boulenger, 1899) and *Neolamprologus brevis* (Boulenger, 1899). The karyotypes of the lamprologini investigated here display 2n = 42 chromosomes. The karyotypes of *A. compressiceps*, *J. transcriptus*, and *N. tetracanthus* are composed of 10 m/sm and 32 st/t (FN = 52) whereas the karyotype of *N. brevis* consists of 14 m/sm and 28 st/t (FN = 56) (Tab. I). All karyotypes of these four species show a single, very large pair of m-sm chromosomes and a single, also large pair of acrocentric chromosomes. In addition, we localized the 18S rRNA genes in the terminal region of short arm of a small st/a chromosome pair in these four species (Fig. 2).

Tribe Limnochromini

Gnathochromis permaxillaris (David, 1936)

The karyotype of *G. permaxillaris*, endemic to Lake Tanganyika, is composed of 2n = 44 chromosomes including 10 m/sm and 34 st/a (FN = 54) (Tab. I). The first metacentric pair and the first acrocentric pair are twice larger than the following ones. The 18S rRNA genes are located in the short arms of a small acrocentric pair (Fig. 2).

Tribe Eretmodini

Eretmodus cyanostictus Boulenger, 1898

The karyotype of *E. cyanostictus,* tribe Eretmodini, endemic to Lake Tanganyika, is composed of 2n = 46 chromosomes including 10 m/sm and 36 st/t (FN = 56) (Tab. I). The first metacentric pair is not much bigger than the other chromosome pairs. The 18S rRNA genes are located on the short arms of a small acrocentric pair (Fig. 2).

Tribe Haplochromini-Tropheini

We sampled the haplochromines *Pseudocrenilabrus multicolor* (Schöller 1903) and *Pseudocrenilabrus philander* (Weber, 1897), *Astatoreochromis alluaudi* Pellegrin, 1904, *Haplochromis paludinosus* (Greenwood, 1980), the endemic species of Lake Malawi basin: *Nimbochromis livingstonii* (Günther, 1894), *Nimbochromis polystigma (*Regan, 1922), *Haplochromis callipterus* (Günther, 1894), and the endemic tropheine species of Lake Tanganyika *Ctenochromis horei* (Günther, 1894).

The following karyotypes could be established: For *A. alluaudi, H. callipterus and H. paludinosus,* 2n = 44 including 12 m/sm and 32 st/t (FN = 56); for *N. polystigma,* 2n = 44 including 14 m/sm and 30 st/t (FN = 58); for *P. multicolor victoriae* and *P. philander,* 2n = 44 including 16m/sm and

28 st/t (FN = 60); for *N. livingstonii,* 2n = 42 including 14m/sm and 28 st/t (FN = 56); for *C. horei,* 2n = 40 chromosomes including 10 m/sm and 30 st/a (FN = 50). For all these species, the first metacentric pair and the first acrocentric pair are twice as large as the following ones.

The 18S rRNA genes are located on the short arms of one acrocentric pair in all species of this clade.

DISCUSSION

Chromosome numbers and formulae

Our results confirm a modal diploid number of 44 chromosomes for the African cichlids karyotyped to date (Feldberg *et al*., 2003; Poletto *et al*. 2010). Nakatani *et al*. (2007) have proposed a diploid number of 2n = 50 acrocentric elements as the ancestral teleost karyotype. The relatively high chromosomal variability displayed by the teleost species karyotyped up to now, involves various rearrangements that include: 1) Pericentric inversions leaving the chromosome diploid number unchanged but increasing the FN; 2) Centric fusions (Hartley and Horne, 1984), producing large meta- or submetacentric elements decreasing the chromosome diploid number but leaving the FN unchanged; and 3) Tandem (centromere-telomere), decreasing the chromosome diploid number and the FN. Therefore cichlid karyotypes composed of 44 chromosomes can arise from both centric and tandem fusions. These three types of chromosomal rearrangements occurred in the species under study.

Madagascar Cichlidae

Subfamily Ptychochrominae

The Ptychochrominae are endemic to Madagascar. According to Sparks and Smith (2004), this subfamily, which includes the genera *Paratilapia*, *Oxylapia*, *Ptychochromides* and *Ptychochromis,* is monophyletic, and is the sister group to the Cichlidae belonging to the East-African clade (Cichlinae + Pseudocrenilabrinae). The Malagasy fishes have been poorly sampled and studied due to difficulties associated with conducting comprehensive ichthyofaunal surveys in Madagascar (Sparks, 2004). Almost all cichlid species of Madagascar are endangered. *Paratilapia polleni* was common and it seems that, in the past, several nominal species have been assigned to this species (for example, *P.* sp. "East coast"). The species is probably a species-complex and needs systematic revision. No chromosomal data were available for the Ptychochrominae and *P. polleni* is the first species to be karyotyped, using animals from an aquarium strain. The karyotype is composed of 44 chromosomes, including a large submetacentric pair, a large acrocentric pair and seven pairs of small m-sm chromosomes, suggesting that the major chromosomal rearrangements in this species involved pericentric inversions in small acrocentric chromosomes.

Karyotype patterns of diversification among African cichlid tribes

Tribe Chromidotilapiini

Chromidotilapia guntheri guntheri belongs to the tribe Chromidotilapiini, the most species rich ancient African cichlid lineage, including 48 described species and 10 genera. This species has a wide distribution throughout West Africa. Their ancient divergence predates the origin of the highly diverse austro-tilapine lineage, comprising the majority of African cichlid species (Schwarzer *et al*., 2015). The karyotype of *C. guntheri guntheri* (2n = 48) is exclusively composed of acrocentric chromosomes. It is very close to the diploid number of 50 acrocentric chromosomes, which is proposed as the hypothetical ancestral chromosome number for teleost fishes (Nakatani *et al*., 2007). The reduction to 48 chromosomes is certainly the result of a tandem fusion between two acrocentric chromosomes, giving rise to the first, big acrocentric chromosome. Its morphology evokes the typical large acrocentric pair observed in almost all species of Tilapiini, and considered as a signature for this tribe (Poletto *et al*., 2010). Available information concerning the karyotypes of Chromidotilapiini is scarce. Only the karyotype of *Pelvicachromis pulcher* (2n = 48) has been published (Post, 1965).

Tribe Hemichromini

Hemichromini are also members of ancient African cichlid lineages and their precise phylogenetic position remains uncertain (Genner *et al*., 2007). *Hemichromis fasciatus,* has a wide West African distribution. Its karyotype (2n = 46) is characterized by a large metacentric pair, which certainly arise from a centric fusion between two acrocentric chromosomes, and a large acrocentric pair, possibly of similar origin as the large acrocentric pair described in *C. guntheri guntheri*. It also shows two pairs of small metacentric chromosomes, arising from pericentric inversions in small acrocentrics. The karyotype of another species of Hemichromini, *H. bimaculatus* (2n = 44), has been published (Poletto *et al*., 2010). This species also possesses a typical, large acrocentric pair, and two large metacentric pairs only. The large acrocentric pair is possibly conserved between the two species. However, both species show different patterns of chromosomal rearrangements. They involve only centric fusion for *H. bimaculatus* and both centric fusions and pericentric inversions for *H. fasciatus*.

Tribe Steatocranini

Steatocranus irvinei (2n = 42), previously assigned to the tribe Gobiocichlini, has been placed in the new tribe Steatocranini, although its generic status remains unclear (Dunz and Schliewen, 2013). This rheophilic species is endemic to the Volta basin. Steatocranini are a sister group to all other haplotilapiine species (sensu Schliewen and Stiassny, 2003; Dunz and Schliewen, 2013), with the exception of the Het-

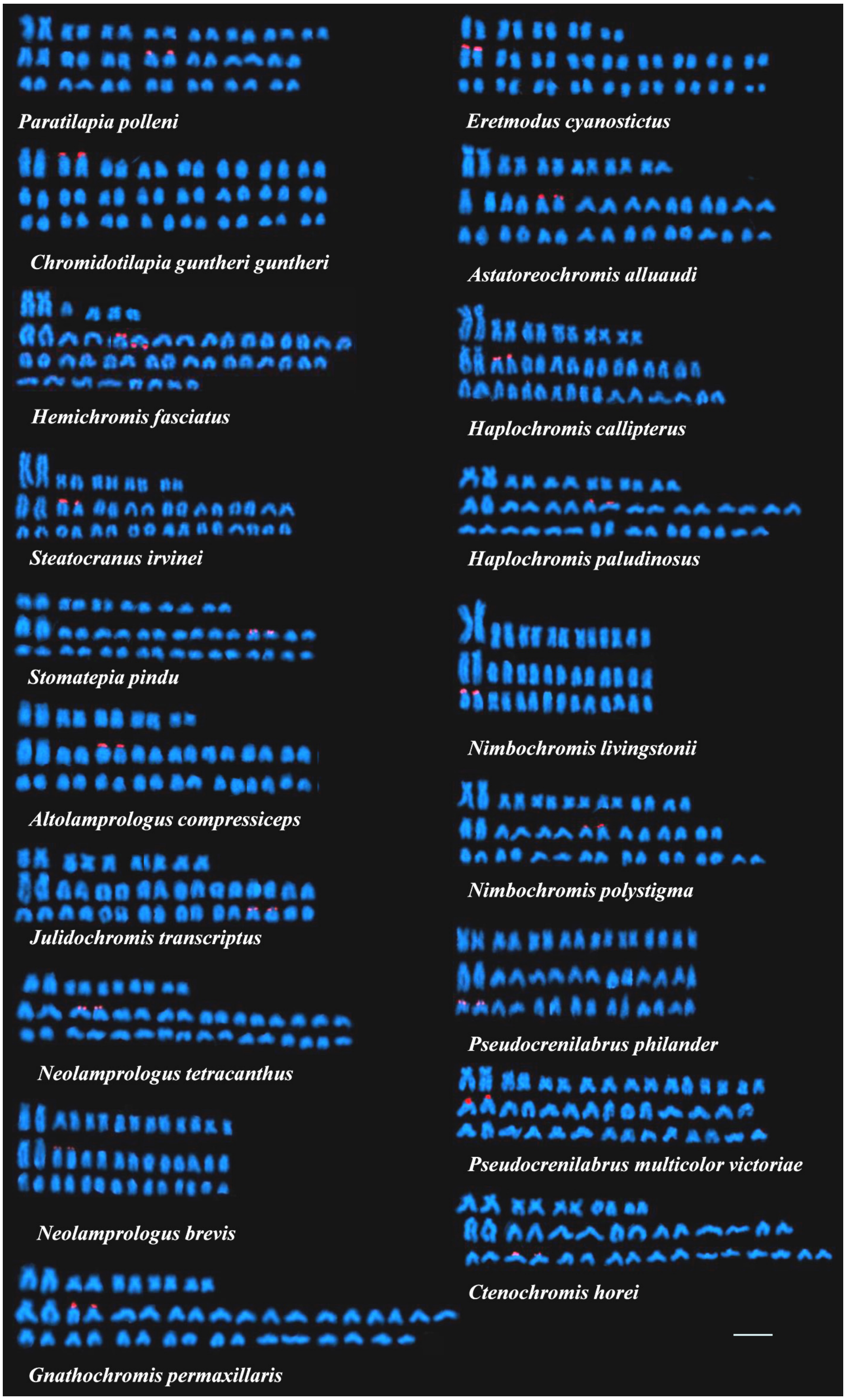
Paratilapia polleni
Eretmodus cyanostictus
Chromidotilapia guntheri guntheri
Astatoreochromis alluaudi
Hemichromis fasciatus
Haplochromis callipterus
Steatocranus irvinei
Haplochromis paludinosus
Stomatepia pindu
Nimbochromis livingstonii
Altolamprologus compressiceps
Nimbochromis polystigma
Julidochromis transcriptus
Pseudocrenilabrus philander
Neolamprologus tetracanthus
Pseudocrenilabrus multicolor victoriae
Neolamprologus brevis
Ctenochromis horei
Gnathochromis permaxillaris

Figure 2. – Karyotypes of Malagasy and African cichlids after conventional fluorescence *in situ* hybridization counterstained with DAPI (blue). m/sm pairs are separated on the first line of each karyotype. The 18S rRNA probed chromosomes are shown (red). Scale bar = 5 μm.

erochromini. Unlike the Tilapiine species karyotyped to date (reviewed and updated in Poletto *et al.*, 2010 and the present work), its first acrocentric pair is only twice as large as the small acrocentrics and probably originates from a tandem fusion between two small elements. Its first chromosome pair in size is a large metacentric probably arising from a centric fusion. It is also characterized by the presence of four small metacentric pairs.

Tribe Tilapiini

The Tilapiini were recently taxonomically revised (Dunz and Schliewen, 2013). *Stomatepia pindu* (2n = 44) is endemic to a crater lake in Cameroon, the Barombi Mbo, and probably speciated independently from other Tilapiini. It is considered closely related to *Sarotherodon* (Schliewen *et al*., 1994). Among the Tilapiini karyotyped to date, it is the only species which does not show a large typical subtelocentric chromosome pair, *i.e*. the first st/t pair of the complement, and probably arises from two tandem fusions involving three small elements (Chew *et al*., 2002; Ferreira *et al*., 2010; Poletto *et al*., 2010). Instead, there is a single medium size acrocentric pair probably arising from a single tandem fusion. *S. pindu* has a noticeably increased number of small m-sm chromosomes, certainly produced by pericentric inversions in small acrocentric pairs.

Tribe Lamprologini

Lamprologini are endemic to Lake Tanganyika, the Congo basin and one is endemic to the Malagarasi River. They are substrate spawners. Four species, belonging to three genera and endemic to Lake Tanganyika, were analysed: *Altolamprologus compressiceps*, *Julidochromis transcriptus*, *Neolamprologus tetracanthus* and *N. brevis*. The diploid chromosome number (2n = 42) is conserved. In all four species, the first acrocentric pair is twice as large as the other acrocentric chromosomes and the karyotype is also characterised by the presence of four to six small metacentric chromosome pairs and one larger submetacentric pair. *N. brevis*, with a significant increase of the fundamental number (FN = 72), possesses six pairs of small metacentrics produced by inversions.

Tribe Limnochromini

The Limnochromini are restricted to deep-water habitats in Lake Tanganyika (Meyer *et al*., 2015). This tribe includes, for now, nine endemic species (Takahashi and Sota, 2016). *Gnathochromis permaxillaris* is the only species karyotyped to date. The karyotype, 2n = 44 chromosomes, is characterized by the presence of a large acrocentric pair, twice bigger as other acrocentrics, a large submetacentric pair and four small metacentric chromosome pairs, as we described for Lamprologini.

Tribe Eretmodini

Eretmodini, "goby cichlids", are endemic and dominate the shallow waters of Lake Tanganyika, together with Lamprologini and Haplochromini. Eretmodini are the sister group to Haplochromini/Tropheini (Meyer *et al*., 2015). We only have at our disposal the karyotype of *Eretmodus cyanostictus* (2n = 46), which is composed of series of metacentrics (five pairs) and acrocentrics of slowly decreasing size) with absence of any large element.

Tribe Haplochromini-Tropheini

Haplochromini and Tropheini have been found to form a monophyletic clade (Nishida, 1991; Meyer *et al*., 2015) and represent the most species-rich tribe of cichlids. They are distributed in lakes and river basins across large parts of Africa, displaying several radiations, including those of Lake Malawi and the Lake Victoria region (Verheyen *et al*., 2003; Salzburger *et al*., 2005; Schwarzer *et al*., 2012; Wagner *et al*., 2012). Most Haplochromini karyotyped by Poletto *et al*. (2010) had 2n = 44 chromosomes, except *Astatotilapia burtoni* (2n = 40), from Lake Tanganyika basin, and showed two typical large chromosome pairs, the first m/sm and the first acrocentric pairs. They were also characterized by the presence of 5-6 small m-sm pairs. The species we had in our samples originate from various lakes and river basins of Africa. All possess 44 chromosomes, except *Nimbochromis livingstoni* (2n = 42). Among them, the species *Astatoreochromis alluaudi*, *Haplochromis callipterus*, *H. paludinosus*, *N. polystigma* and *N. livingstoni* show similar karyotypes, with these two typical large chromosome pairs. The Tropheine species *Ctenochromis horei* (2n = 40) also possesses a relatively larger m-sm pair and a rather large acrocentric pair in comparison with the other elements composing the karyotype. In contrast, the riverine species *Pseudocrenilabrus multicolor victoriae* and *P. philander* karyotypes are more comparable with the karyotype of *Eretmodus cyanostictus,* with absence of significantly large chromosome pair.

Chromosomal localization of the 18S rRNA genes

In agreement with Nakajima *et al*. (2012), we localized the 18S rRNA gene clusters in terminal position of the p arm of a small acrocentric pair, in the Malagasy species and in almost all African species except in *H. fasciatus*. In the latter, we consistently found the 18S rRNA clusters in two small acrocentric chromosomes of similar sizes, one in the p arms and the other one in terminal position in the long arms. Since we analysed only one specimen, we cannot decide, at this stage, whether these are population or species differences. It can result from the occurrence of a pericentric inversion involving the major rDNA segment resulting in a heterozygous condition for the NOR-bearing chromosome. This can be a cause for a pairing disturbance and further studies about the inheritance of this chromosome would be necessary on a wider sample.

In the present study, we have also noticed that the relative size of the NOR-bearing pairs, compared to the rest of the chromosome set, fluctuate from one species to another. This suggests that this single NOR-bearing pair of small acrocentric chromosomes is possibly not conserved among African cichlids, although it is widely observed and considered as the ancestral hypothetical condition (Feldberg *et al*., 2003). In other cichlid species karyotyped until now, NORs (localized by FISH) have been found in this position except in *O.*

niloticus (Martins *et al*., 2000; Poletto *et al*. 2010; Nakajima *et al*., 2012).

CONCLUSION

Our results confirm a modal number of 2n = 44 for African cichlids. Haplochromine species karyotypes can be characterized by the presence of two typical large acrocentric chromosome pairs, one m-sm and one st-t (as suggested by Poletto *et al*., 2010), but with the exception of the two *Pseudocrenilabrus* species, which lack the presence of these large chromosomes. However, we could also observe the presence of two similar typical large chromosomes in *G. permaxillaris* (Limnochromini), in the four species of Lamprologini, and in the two Hemichromini species, *H. fasciatus* and *H. bimaculatus*. Molecular techniques, such as chromosome painting or CGH (comparative genomic hybridization), would be necessary to verify whether these typical pairs are syntenic across the species/tribes where they have been observed. The presence of a first large chromosome acrocentric pair probably characterizes Tilapiine species. However, in *S. pindu* and *S. irvinei,* this large acrocentric element is relatively smaller than in the Tilapia group, and more likely arises from a single tandem fusion and not from two tandem fusions like, for instance, in the Nile Tilapia *O. niloticus* (Chew *et al*., 2002). Interestingly these two species are endemic to a crater lake, the Barombi Mbo crater in Cameroon, and may have undertaken another pathway of chromosomal speciation.

Three types of chromosomal rearrangements occurred during the rapid diversification of the African Cichlidae, involving few tandem or centric fusions and a more or less important proportion of pericentric inversions. Only molecular cytogenetics using FISH with combinations of several markers and/or chromosome painting would substantiate whether all these rearrangements occurred independently or if some of them represent synapomorphies for particular cichlid groups.

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